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PCR Amplification and RFLP Analysis of Meca Gene Isolated from MRSA

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Abstract

Methicillin Resistant Staphylococcus aureus (MRSA) are posing as a prominent nosocomial infection all over the world because of their rapid development of antibiotic resistance due to their extreme adaptability to antibiotic pressure .the objective of the study was to characterize the strains phenotypic and genotypic method.

In the present study, out of 30 Staphylococcus aureus isolates were isolated from different clinical samples, from which15 strains were identified as MRSA. Among 15 MRSA 6 highly resistant strains were amplified and had 200bp product. The amplified products were subjected to the digestion with Hind II. All the strains showed a pattern of restriction of two bands indicating a single restriction site for Hind II restriction endonuclease in the mecA gene.

This study shows that mecA RFLP can be used as a tool for classification of MRSA strains, also details concerning the genetic diversity that exists in the population of Staphylococcus aureus isolates thus helping in judicious use of antibiotics.

Key words: MRSA, antibiotic resistance, PCR-RFLP and mecA

Introduction

Methicillin Resistant Staphylococcus aureus (MRSA) are posing as a prominent nosocomial infection all over the world because of their rapid development of antibiotic resistance due to their extreme adaptability to antibiotic pressure [1, 2]. Proper typing and accurate identification of diseases causing agent is prerequisite for disease control. This PCR-RFLP typing method is an attractive tool in routine epidemiological surveillance. Its impressive characteristics are ease of performance and interpretation, while at the same time guaranteeing good discriminatory power, reproducibility, and type ability. RFLP is a technique in which organisms or even strains may be differentiated by analysis of patterns obtained from cleavage of DNA by restriction enzyme.

Materials and Methods

Isolation of S. aureus from different clinical sample

Sample collection

In the present investigation the clinical samples were selectively collected from patients from private diagnostic center. The clinical sample like cervical swab, chronic sinusitis, urine, pus, blood, sputum, cerebrospinal fluid pleural fluid and stool etc were collected every alternate day in sterile borosil screw capped bottles containing peptone water as a transport media. The samples thus collected were immediately transferred to laboratory for further analysis. The samples thus collected were immediately transferred to laboratory for further analysis. BHI Agar was used to enrich the Sample. Nutrient Agar, Baird Parker Agar with Egg Yolk Emulsion and Potassium Tellurite, Mannitol Salt Agar, Blood Agar was used for identification and characterization of *S. aureu*s.

S. aureus ATCC 2593 and ATCC 29737 (NCIM 5021 and 2901 respectively obtained from National collection of Industrial Microorganisms (NCIM) National Chemical Laboratory Pune, were used as standard control organisms in all biochemical characterization.

Screening of multidrug resistant pathogenic S.aureus through antibiogram Antimicrobial susceptibility patterns were determined according to the Clinical and Laboratory Standards.Institute (CLSI)-recommended modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar with commercial antibiotic discs.

Molecular chaterisation

Isolation of genomic DNA

Overnight culture was collected for DNA isolation.The isolated genomic DNA was subjected to proteinase k and RNase treatment in order to remove protein and RNA contamination. The purity of the genomic DNA was confirmed by agarose gel electrophoresis.

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PCR Amplification of mecA gene

Amplification of mec A gene from genomic DNA was carried out by polymerase chain reaction (PCR). The 21 base pairs oligonucleotide primer pair was synthesized from Genei Pvt Ltd, Bangalore, India. The primer pair and their sequences are as follows. Forward CCA ACT GTC GTA GTC GAA ACC Reverse CTA AGG CAC AAA AAT GGT [3-5].

The Genomic DNA was isolated from MDR strains of S. aureus and was diluted to concentrationof 20 ng/µl. The PCR amplification was performed according to Oliveira et al.,2002 [8] with some modifications. The content in the tube were mixed thoroughly and over laid with 20 µl of mineral oil to prevent evaporation. The PCR amplification programme was standardized for 35 cycles and performed using following conditions. The above amplified product was subjected to 2% (W/V) agarose gel electrophoresis with 200 bp DNA as molecular weight marker and electrophoretic profile was documented using photo gel documentation system [7,10,11] and photograph was printed using thermal printer.

Restriction of amplified DNA

Preparation of reaction mixture

Adding following reagents to PCR product for restriction digestion. The reaction mixture was centrifuged for few seconds and incubated at 37° C for 2 hrs. After incubation 5ml of loading (6X) dye is added to stop the reaction. The tubes are spin for 2 minutes. Electrophoresis was carried out to visualize the restriction pattern.

Result

In the present study, 30 S. aureus strains were isolated from total of 55 clinical samples (Table 1); they were identified and screened on mannitol salt agar selective media.As per the results on the biochemical tests all the strains were beta hemolytic and coagulase positive.

Distribution od S. Aureus isolates in clinical samples	
Source	No. of Strains isolated
Pus	4
Blood	10
Urine	15
Pleural Fluid	1
Total	30

Table 1: Distribution of S. aureus isolates in clinical samples

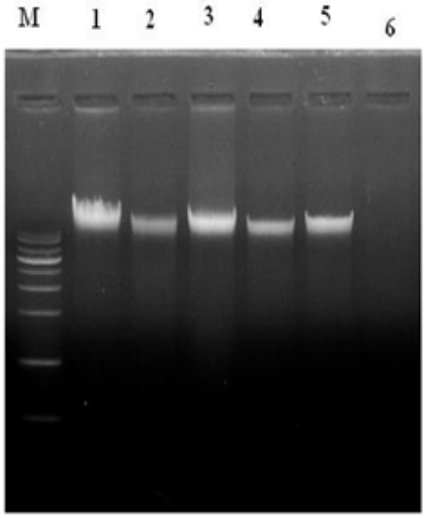
The antibiotic profiles of all 30 clinical S. aureus isolates examined are shown in table 2. S.aureus isolates have shown significant rate of antibiotic resistance to various antibiotic useable . On the whole 17 strains i.e., 56% isolates were MDR strains and 50% strains were MRSA. The results reveal that half of the strains isolate from clinical samples are multi drug resistant.

Out of 30 isolates, 15 strains were MRSA. Out of the 15 MRSA strains, 6 MDR strains were used for the amplification of mecA gene (200bp) as shown in the Figure 1 (A,B,C,D) . All the strains were positive for the amplification studies with specific primers used as mentioned earlier. The remaining isolates were negative for the mecA amplification indicating that phenotypic antibiotic resistance did not match with genotypic antibiotic resistance.

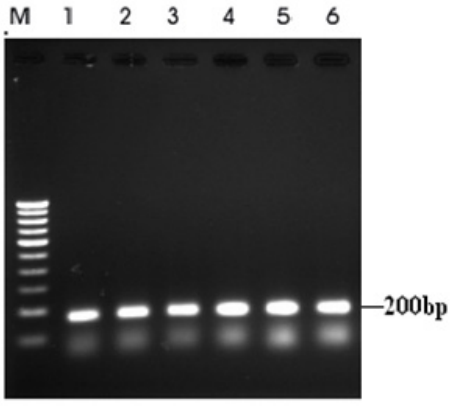
Antibiotics	No of resistant strains	Percentage of resistance
Amplicillin (Amp)	25	83
Ciprofloxacin (Cf)	18	60
Erythromycin (E)	16	53
Gentamycin (G)	10	30
Methicillin (M)	15	50
Oxacillin (Ox)	7	23
Rifampicin (R)	18	60
Streptomycin (S)	8	26
Torbramycin (T)	17	56
Vancomycin (V)	3	10

Table 2: Antibiotic resistance rate among the S. aureus isolates

Figure 1: PCR amplification of mecA gene



A.Genomic DNA of Methicillin Resistant,S. aureus; Lane M-100bp DNA ladder; Lane 1-6- Isolated genomic DNA



B. Lane M-100bp DNA ladder
Lane 1-6- amplified mecA gene of 200 bp

Process	Temperature (in °C)	Time (in min.)	Cycles
Initial Denaturation	94°C	2 minutes	1
Annealing	54°C	1 minutes	
Extension	72°C	2 minutes	
Denaturation	94°C	1 minutes	35
Annealing	54°C	1 minutes	
Extension	72°C	2 minutes	
Final extension	72°C	8 minutes	1
Storage	4°C	Forever	

C. Program for mecA gene PCR amplification

Constituents of PCR mixture		
constituents	Quantity	Final Concentrations
Template DNA	2.0 ml	20 ng
10X Taq DNA assay buffer	2.0 ml	1X
DNTPs	0.4 ml	25 mM each dNTPs
Primers Forward & Reverse	2.0 ml	1.0 mM each
XT-Taq DNA polymerase	0.3 ml	1 Unit
Sterile water	11.7 ml	20 ml

D. PCR amplification of mecA gene

Using the PCR-RFLP method, we examined the polymorphism of mecA gene. During this study we determined the mecA genotypes by Hind-II PCR-RFLP for five Staphylococcal strains. All the strains showed a pattern of restriction of 2 bands on agarose gel of molecular weight 150bp and 70bp indicating a single restriction site for Hind-II restriction endonucleases in the mecA gene (Figure 2).

Discussion

This PCR-RFLP is the an attempt of investigation of antimicrobial susceptibility pattern among thee MRSA isolates of this Gulbarga region. There are many advantages of genetic susceptibility methods when compared to other conventional susceptibility methods.The high incidence of MDR (56%) and MRSA (50%) strains can ascribed to the fact that patients hah been on several anti microbial agents treatments before the strains were isolated [1].

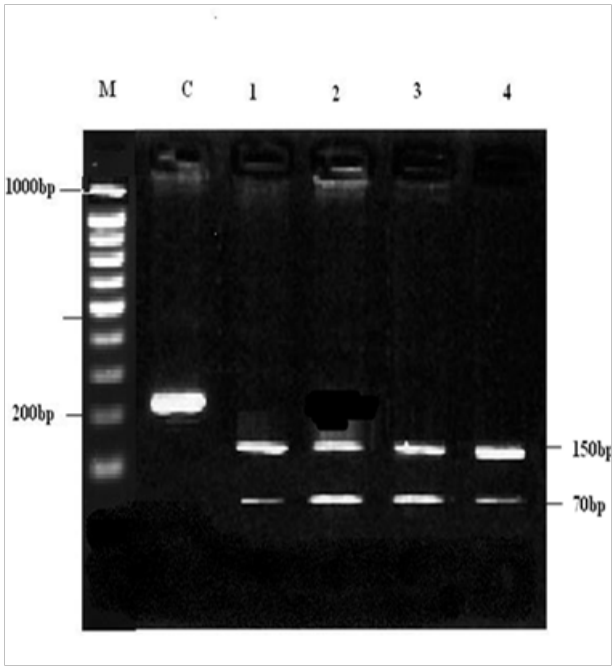


Figure 3. Restriction digestion reaction mixture
Lane M-100bp DNA ladder
Lane C- Control mecA amplified product
Lane 1, 2, 3, 4- Restriction digestion product by enzyme Hind II

Such MRSA were of significance globally, more recently in India also have been major cause of nosocomial infection . The emergence of Staphylococcus aureus strains resistant to multiple antibiotics has made the treatment of Staphylococcal infections more problematic tedious and nightmare to clinicians.

This study demonstrates the novel PCR-RFLP of mecA gene as a rapid typing method for MDR strains. We confirm that the PCR-RFLP method is highly specific time effective simple and can be achieved with just one pair of primers and two-three restriction enzymes These results suggest that the PCR-RFLP assay provides rapid, accurate, and reliable species-level identification of Staphylococcus aureus [6-7, 9].

Conclusions

The emergence and spread of methicillin resistant Staphylococcus aureus (MRSA), both as nosocomial pathogen and more recently in community are matters of concern worldwide. Emergence of methicillin and multi drug resistant staphylococcus aureus is a worrying development and has made the treatment more problematic to clinicians. Detection of polymorphism at DNA level is usually based on deletion, addition, inversion and transmutation of DNA fragments and even single base pair change which leads to differential amplification of DNA. This will help in understanding the diversity of bacterial species, DNA fingerprinting, gene mapping etc along with drug resistance analysis. By using a series of control strains in each experiment the assay will be easy to standardize and interpret.

Acknowledgments

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RFLP assay provides rapid, accurate, and reliable species-level identification of Staphylococcus aureus [6-7, 9].The authors are (Prabhurajeshwar C and Kelmani Chandrakanth) profusely thankful to the Department, Gulbarga University, Gulbarga for providing facilities for pursuing the research work at the Department.

Conflict of interest

We declare that no conflict of interest.

Authors Contribution

First authors are responsible for carrying out the research work, data analysis and optimization of experimental work and Corresponding author is responsible for research planning executing and providing valuable inputs and in writing manuscript.

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